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REGULAR ARTICLE

# AtMYB20 is negatively involved in plant adaptive response to drought stress

**Shuai Gao · Yong Li Zhang · Lu Yang · Jian Bo Song · Zhi Min Yang**

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**Abstract**
*Background and aims* MYB transcription factors play critical roles in plant development and stress responses. Our objective was to characterize a role of *AtMYB20* (AT1G66230) in regulating the ABA-dependent adaptive response to desiccation stress in Arabidopsis.
*Methods* Sequencing analysis revealed that there is a site located on the *AtMYB20* transcript which is potentially base-paired by miR858. To avoid the possible cleavage, a vector with a miR858-resistant version of *AtMYB20* under the CaMV 35S promoter (*35S:m5AtMYB20*) was constructed. The *AtMYB20* knock-out mutant *myb20* was applied to identifying *AtMYB20* functions.
*Results* While *AtMYB20* was induced by high levels of NaCl, its expression was suppressed by desiccation and cold stresses and abscisic acid (ABA) treatment. Compared with wild-type, *AtMYB20* over-expression (*35S:m5AtMYB20*) seedlings were susceptible to desiccation, whereas MYB20 loss of function mutant *myb20* plants displayed resistance to desiccation stress. *35S:m5AtMYB20* plants were less sensitive to ABA, but *myb20* mutants were hypersensitive to ABA. This could be validated by the experiment in which treatment with 10 μM ABA for 2 h resulted in constant stomatal opening on leaves of *35S:m5AtMYB20* plants but stomatal closure on *myb20* mutant plants. Expression of ABA- and drought stress-responsive marker genes (*e.g. ABI3-5* and *ABF3-4*) was up-regulated in *myb20* plants but down-regulated in *35S:m5AtMYB20* plants.
*Conclusions* *AtMYB20* acts as a negative regulator of plant response to desiccation stress in an ABA-dependent manner.

**Keywords** Arabidopsis · *AtMYB20* · Desiccation · Abscisic acid

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Shuai Gao and Yong Li Zhang made equal contribution to the study

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## Introduction

Plants grow in changing environments and their growth and development are constantly influenced by environmental stresses such as desiccation (or drought), salt, cold, high or low temperature, and even heavy metals (Khraiwesh et al. 2012; Yang and Chen 2013). Water deficit is one of the most serious environmental problems that influence the worldwide plant growth and crop production. Under drought stress, biochemical and physiological metabolisms are altered, and cellular aqueous and ionic equilibriums are disrupted. Furthermore, hundreds of genes at transcriptional and post-transcriptional levels are reprogrammed (Sreenivasulu

et al. 2007; Chen et al. 2012; Zhou et al. 2013). Plants have evolved delicate mechanisms to cope with drought stress, and their physiological and molecular changes are usually considered as adaptive responses to the adverse environment (Zhu 2002; Shinozaki et al. 2003). To date, a number of drought-inducible genes have been characterized, and most of them are found to be involved in synthesis of dehydrins and osmolytes, enzymes for anti-oxidation, and components for ABA synthesis or response (Bartels and Sunkar 2005). In addition, a large group of specific genes coding for transcription factors (TFs) are shown to be activated by drought stress (Singh et al. 2002; Zhu 2002). In plants, the TFs have been classified into different superfamilies, *e.g.* MYB, bZIP, WRKY, NAC and AP2, and some of members from the families participate in various plant responses to abiotic stresses (Riechmann et al. 2000).

Plant MYB TFs belong to one of the largest TFs families (Chen et al. 2006). Currently, more than 100 MYB TFs have been found in Arabidopsis, rice (*Oryza sativa*), and other plant species (Cedroni et al. 2003; Shinozaki et al. 2003; Rahaie et al. 2010; Liu et al. 2011). According to the number of adjacent imperfect repeats (51–53 amino acids) in their DNA-binding domain, MYB transcription factors can be classified into several subfamilies including R1/2-MYB, R2R3-MYB (3R-MYB) and 4R-MYB (with four R1/R2-like repeats) (Dubos et al. 2010). Recently, several MYB TFs have been reported to involve plant response to drought stress. R2R3-MYB proteins such as AtMYB60 and AtMYB96 (subgroup 1) are involved in this response. *AtMYB60* and *AtMYB96* regulate plant drought stress response through the ABA signaling cascade (Cominelli et al. 2005; Seo et al. 2009). Also, both *AtMYB2* and *AtMYB44* were identified as regulators of ABA-dependent salt and drought stress responses (Abe et al. 2003; Jung et al. 2008). Despite the existence of numerous R2R3-MYB protein-encoding genes, only a few of them have been functionally identified, particularly with regard to the plant drought stress response. In this study, we genetically identified an Arabidopsis gene coding for AtMYB20 (R2R3-MYB protein) transcription factor and show that *AtMYB20* is involved in plant response to desiccation stress in the plant. *AtMYB20* overexpression (*35S:m5AtMYB20*) plants show sensitivity to desiccation, whereas *MYB20* loss of function *myb20* mutant plants display resistance to desiccation stress. *35S:m5AtMYB20* plants showed ABA resistance phenotypes, but *myb20* mutant plants were hypersusceptible to ABA during the process of desiccation. These results indicate that *AtMYB20* is negatively involved in plant adaptive response to drought stress.

## Materials and methods

### Plant materials and treatments

Arabidopsis ecotype Col-0 was used throughout the study. The *myb20* T-DNA insertion mutant (SALK_CS304073) seeds were obtained from ABRC (Ohio State University, Columbus, OH). Seeds were germinated on Murashige and Skoog (MS) solid medium with 1– 3 % Suc and 0.8 % phytoagar (pH 5.7) in a growth chamber at 22 °C with 100 μE $m^{-2}$ $s^{-1}$ photosynthetically active radiation and a 16 h light/8 h dark cycle for 7 days. After that, the seedlings were transferred to a black polyvinylchloride pot (1 L) containing the 1/2-strength Hoagland nutrient solution. Each pot contained six seedlings, which grew for another 7 days under the same condition. Normally, 2 weeks-old seedlings were used for stress treatments. For water loss determination, rosette leaves of the 2 weeks-old plants were detached, placed on an open-lid Petri-dish (dehydrated on filter paper) at room temperature and weighed at a certain period of time after their excision. Water loss was calculated as the percentage of initial fresh weight.

To identify the response of transgenic plants to drought stress, approximate 40 wild type seeds were sowed on the half surface of a pot with substrate, and the same number of *myb20* or *35S::m5MYB20* seeds were sowed on the left site of the pot. The substrate used to culture Arabidopsis composed of humus soil, vermiculite and perlite in the ratio of 2:1:1 (Du et al. 2012). The experiments were repeated thrice. Each experiment was carried out with three treatments and each treatment contained at least three pots. After germination, seedlings grew for 2 weeks. Before the experiment was initiated, the substrate was saturated with water (with the maximum water-hold capacity). The seedlings were then not watered for 21 days. Photographs were taken 3 days after rewatering (Zhang et al. 2007; Li et al. 2012). Seedlings were irrigated through the bottom of a pot. Drought tolerance was assayed as the ability of plants to resume growth when returned to normal conditions.

For salt treatment, seedlings were transferred to the half-strength Hoagland nutritional solution with NaCl. The high concentration of NaCl (300 mM) used in this study

was set based on the method described previously (Zhang et al. 2007; Li et al. 2012). For cold treatment, seedlings were moved to a chamber with temperature at 4 °C. ABA treatment was undertaken by spraying solution on leaves. The harvested plants were immediately frozen in liquid nitrogen and stored at −80 °C for analysis.

### Plant transformation

The plasmid construction and plant transformation were performed based on the method described previously (Clough and Bent 1998). To construct the *35S:m5AtMYB20* transgenic plants, two sequences were PCR-amplified from *AtMYB20* cDNA with the pairs of primers M5-s/T-a and T-s/M5-a (Supplementary Data 1A), respectively. With the two PCR products as common template, the miR858-resistant version of *AtMYB20* was amplified by overlapping extension PCR using the primer pair of M5-s/M5-a, and cloned into the *BglII*/*SpeI* sites of the binary vector pCAMBIA1304. This vector was used as the plant expression vector with CaMV35S as a promoter and NOS terminator as transcriptional termination sequences (Shen et al. 2011). All amplified DNA was sequenced and confirmed. The vector was transformed into *Agrobacterium tumefaciens* strain LBA4404 and transformed into Arabidopsis using the floral dip method (Song et al. 2012). In this study, all homozygous transgenic lines (T4 generation) were used.

### RT-PCR analysis

Real time-PCR and semi-quantitative PCR were performed to analyze gene transcripts based on the methods described previously (Guo et al. 2008; Shen et al. 2011). Briefly, total RNA was isolated by the method indicated above, and 1.0 μg RNA was used as templates for cDNA synthesis. Quantitative RT-PCR (qRT-PCR) was conducted on CFX96 Real-Time PCR Detection System (Bio-Rad). Amplification reaction was performed in a 25 μL mixture containing 5 ng template, 12.5 μL SYBR-Green PCR Mastermix (Toyoba, Japan) and 10 pmol primers. The temperature profile was 98 °C for 30s, followed by 40 cycles at 98 °C for 2 s, 60 °C for 5 s and melt curve at 65 °C for 5 s. Data were analyzed using CFX Data Analysis Manager Software. The relative expression level was normalized to *ACTIN2*, which was used as the internal control, with the $2^{-\triangle CT}$ method representing the relative quantification of gene expression. The primers used for analysis are presented in Supplementary Data 1B.

### Stomatal aperture measurement

Stomatal apertures were determined in the focal planes of the outer edges of guard cells in epidermis (Lemichez et al. 2001). Detached leaves of 4 weeks-old seedlings were incubated in the stomatal opening solution with 10 mM KCl, 100 mM $CaCl_2$, and 10 mM MES (pH 6.1) for 2 h, and transferred to the same solution with ABA at 0, 1 and 10 μM for 2 h. Subsequently, the adaxial surface of each leaf was applied to 3 M clear tape to peel off the epidermal layer. The epidermal strips were mounted on glass slides and observed with a microscope (YS100, Nikon). Photographs were taken with a digital camera (P5000 COOLPIX, Nikon) attached to the microscope. The ratio of width to length of the stomata was measured using Multigauge version 3.1 soft ware (Fuji Film). More that 60 guard cells from each sample were monitored.

### Statistical analysis

All experiments in the study were independently performed three times.Each result shown in the figures was the mean of at least three replicated treatments and each treatment contained at least 12-40 seedlings. Unless indicated, the equal amount of mixed transgenic line seeds was used and samples for analysis were randomly selected from all transgenic lines. The significant differences between treatments were statistically evaluated by standard deviation and one-way analysis of variance (ANOVA). The data between differently treated groups were compared statistically by ANOVA, followed by the least significant difference (LSD) test if the ANOVA result was significant at $P<0.05$.

## Results

### Expression of *AtMYB20* under abiotic stresses and ABA treatment

To identify whether *AtMYB20* expression responded to abiotic stresses, 2 weeks-old Arabidopsis seedlings were exposed to desiccation, salt and cold for 1–12 h, depending on the treatments. Total RNA was isolated from seedlings. We first tested the expression of *RD29A*, a stress responsive gene (Shinozaki et al. 2003), as a

positive control. Semi-quantitative RT-PCR analysis revealed that *AtMYB20* could be induced by NaCl, but suppressed by desiccation and cold (Fig. 1a). To validate the observation, a quantitative real-time RT-PCR experiment was carried out. *AtMYB20* had a similar expression pattern in response to the stresses (Fig. 1b, c). Expression of *AtMYB20* was induced 3.5-fold by NaCl, while expression of *AtMYB20* in response to desiccation and cold was only 37–47 % and 22–55 % of the control, respectively. The phytohormone ABA mediates the plant response to various abiotic stresses (Zhu 2002). Examination of *AtMYB20* response to ABA treatment (100 μM) demonstrated that *AtMYB20* was also depressed. Its expression was 23–27 % of the control (Fig. 1b). The expression of control genes (RD29 and RAB18) was confirmed for *AtMYB20* expression. These results indicate that *AtMYB20* can be differently regulated by abiotic stresses.

## Identification of *35S::m5AtMYB20* and *myb20* mutant lines

To identify the role of *AtMYB20* in desiccation response, we constructed transgenic plants over-expressing *AtMYB20* driven by the cauliflower mosaic virus (CaMV) 35S promoter. Because *AtMYB20* was identified as one of the potential targets of miR858 (German et al. 2008), a miR858-resistant version (*35S::m5AtMYB20*) was generated by introducing five silent mutations in the miR858 binding site but without changing the protein sequences (Fig. 2a). The vector was transformed into Arabidopsis. The transgenic lines can over-express *AtMYB20* (*35S::m5AtMYB20*) without cleavage of its mRNA by miR858. The *35S::m5AtMYB20* transgenic lines were screened and identified from T0 to T4 generation. Finally, a total of three homozygous *35S::m5AtMYB20* transgenic lines

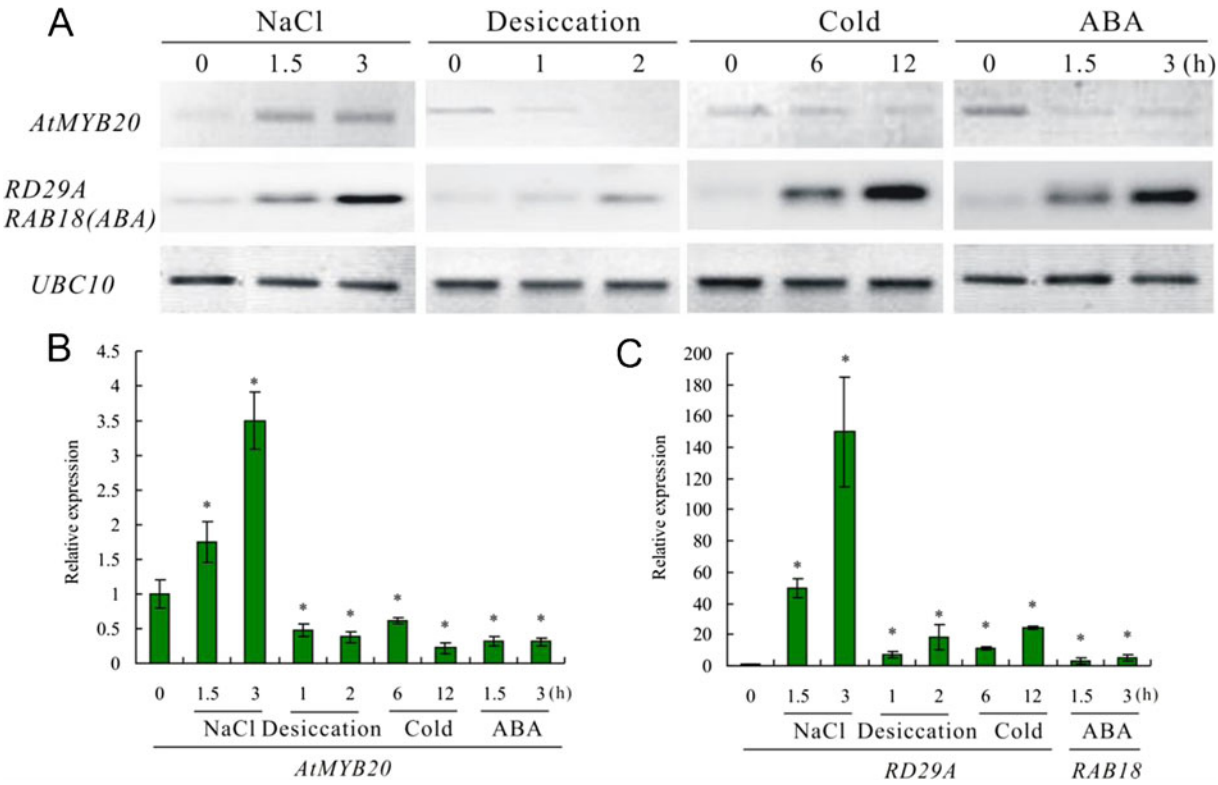


**Fig. 1** Expression of *AtMYB20* in response to abiotic stresses and ABA treatment. Two weeks-old wild-type Arabidopsis seedlings were treated with NaCl (300 mM, for 1.5 and 3 h), desiccation (for 1 and 2 h), cold (4 °C for 6 and 12 h), and ABA (100 μM, for 1.5 and 3 h). After treatments, the expression of *AtMYB20* was analyzed by semi-quantitative RT-PCR (**a**) and quantitative real-time PCR (**b** and **c**), respectively. Expression of genes *RD29* and *RAB18* was used for the positive control. *Vertical bars* represent SD of the mean of three treatments. *Asterisks* indicate that mean values are significantly different between the treatment and control ($p<0.05$)

have been obtained. The transgenic plants carrying *35S::m5AtMYB20* have 4.6 to 6.8-fold *AtMYB20* expression levels over the wild-type (WT) (Fig. 2b).

The genomic sequences of *AtMYB20* contain 925 bp, comprising two exons (849 bp), which are interrupted by an intron towards 5′ end; the CDS contains an open reading frame coding for a protein with 282 amino acid residues (Fig. 3a). A T-DNA insertion mutant, SALK_CS304073 (*myb20*) was obtained from the Arabidopsis Biological Resource Center. The mutant *myb20* has a T-DNA insertion in the second exon towards 3′ UTR. The mutant was verified by diagnostic PCR using *AtMYB20* gene-specific and T-DNA border primers. So there was no full length of *AtMYB20* transcripts to be expressed (Fig. 3b).

## *AtMYB20* regulates plant response to dehydration stress

Two weeks-old wild-type, *myb20* and *35S:m5AtMYB* plants cultured in soil were placed under desiccation treatment for 3 weeks, and most of the plants were withered (Fig. 4a). After a 3-day re-watering, 85.7 % of *myb20* mutant plants displayed continued survival and growth, whereas only 45.4 % of the wild-type plants survived (Fig. 4b). By contrast, the *35S::m5AtMYB20* plants showed a more severe dehydrated phenotype. After re-watering for 3 days, there was only 42.2 % survival (Fig. 4c). These results indicated that the *35S::m5AtMYB20* plants were more sensitive to desiccation stress than *myb20* mutant plants.

We further examined the plant response to desiccation stress using the method of water loss rate (Bouchabke et al. 2008). The detached rosette leaves of 2 weeks-old wild-type, *myb20*, *35S::m5AtMYB20* plants were placed on the open-lid petri dishes under the dim light at room temperature. The reduced fresh weight was measured over time (0–330 min). Rosette leaves from all plants showed progressive loss of weight (Fig. 4d). To the end of the experiment, the fresh weight for *myb20* mutant leaves was 50.4 %. The fresh weight for *35S::m5AtMYB20* plant leaves was only 28.7 % of their starting weight, and the wild-type had an intermediate value of 34.6 %. Although the *35S::m5AtMYB20* plants contained less water in leaves than wild-type at the end of experiment, no significant difference was found between them.

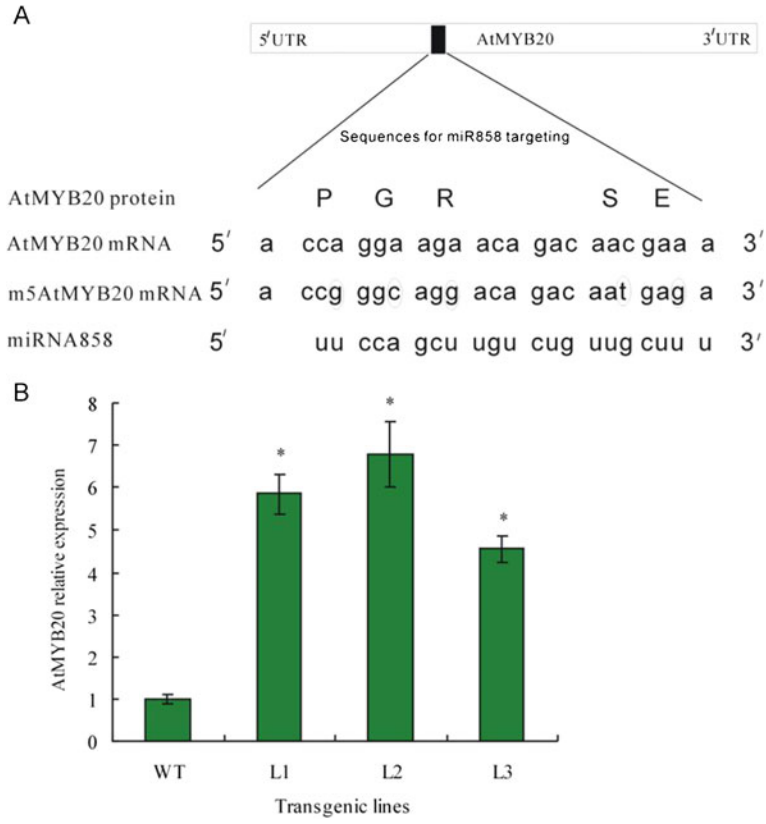


**Fig. 2** Base paring of miR858 with its corresponding complementary site of *AtMYB20* and construction of a miR858-resistant form of *AtMYB20* (*m5AtMYB20*) (**a**) and expression of *AtMYB20* in *35S:m5AtMYB20* transgenic lines (**b**). Seedlings were grown hydroponically for 14 days. Total RNAs were extracted from plants. Quantitative real-time RT-PCR assays were carried out (**a**, **b**). *Vertical bars* represent SD of the mean of three treatments. *Asterisks* indicate that mean values are significantly different in *AtMYB20* expression between the transgenic lines and wild type (WT) ($p<0.05$)

*AtMYB20* is involved in ABA-dependent stomatal closure

Leaves of 4 weeks-old plants were submerged in a stomatal opening solution and treated with 10 μM ABA for 2 h. In the absence of ABA, all guard cells on the leaves of wild-type, *35S::m5AtMYB20* and *myb20* plants were fully opened in the stomatal opening solution (Fig. 5a). However, when ABA was added to the solution, the stomata on the leaves of *myb20* plants were closed, whereas those on *35S::m5AtMYB20* plant leaves were still open. Quantitative analysis using stomatal aperture (the ratio of width to length) showed that the much stronger stomatal closure occurred in the *myb20* plants than in the wild-type with ABA (Fig. 5b). Conversely, stomata on the *35S::m5AtMYB20* leaves were not closed but a little more open than those of WT.

Expression of desiccation- and ABA responsive genes in *35S::m5AtMYB20* and *myb20* plants

To investigate further whether the response of desiccation stress could be regulated by *AtMYB20*, several abiotic stress and ABA responsive genes were analyzed. *RD22*, *RD29A*, *KIN1* and *COR47* belong to the DRE/CRT (drought responsive/C-repeat) elements-containing class of stress- and ABA-responsive genes (Yamaguchi-Shinozaki and Shinozaki 1993; Shinozaki et al. 2003). In the presence of ABA, *RD22*, *RD29A*, *KIN1* and *COR47* were expressed at higher levels in *myb20* mutant than in wild type plants (Fig. 6a–d). Compared to wild type, expression of *RD22*, *RD29A*, *KIN1* and *COR47* was increased 5.8, 2.5, 2.4 and 1.6 folds, respectively. By contrast, expression of these genes was lower in *35S::m5AtMYB20* plants. We further analyzed three Ser/Thr protein phosphatase 2C genes *ABI1*, *ABI2* and *AtPP2CA*. These genes have been characterized as negative regulators of ABA signal (Schweighofer et al. 2004) and respond to salt stress (Cui et al. 2013). Our analysis showed that expression of *ABI1*, *ABI2* and *AtPP2CA* was significantly reduced in *35S::m5AtMYB20* plants but significantly increased in *myb20* mutant plants with ABA treatment (Fig. 6e–g). This pattern was very similar to *RD22*, *RD29A*, *KIN1* and *COR47* in *AtMYB20* over-expression and *myb20* mutant plants, respectively. Finally, we analyzed the genes *ABI3*, *ABI4*, *ABI5*, *ABF3*, and *ABF4*. This group of genes encodes ABA-responsive basic leucine zipper (bZIP) transcription factors which bind to the ABA response element (ABRE) of their targets and function during the plant stress responses (Hauser et al. 2011). After treatment with 100 μM ABA for 5 h, all the genes were induced in *myb20* mutant plants, although the degree of the gene induction varied differently (Fig. 6h–l). Also, expression of the genes was lower in *35S::m5AtMYB20* plants. Overall, *AtMYB20* over-expression resulted in the transcriptional modulation of desiccation, salt- and ABA-responsive genes.

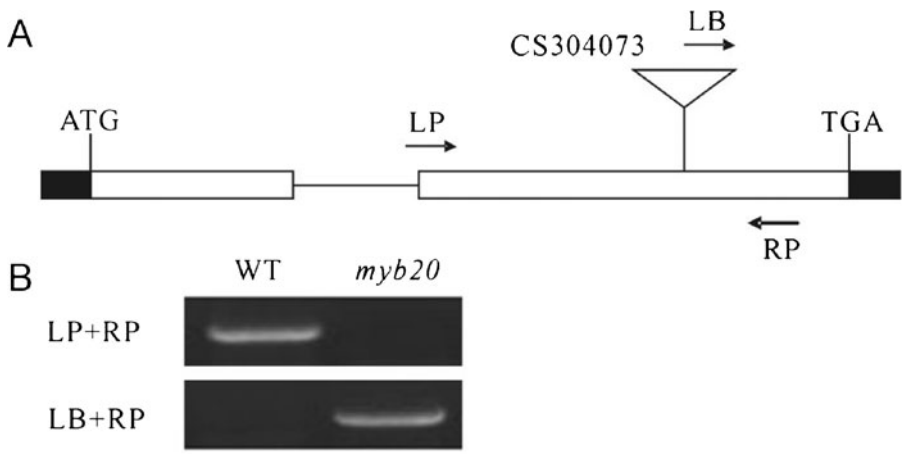


**Fig. 3** Molecular characterization of *myb20* mutants. **a** a schematic structure of the *myb20* with the T-DNA insertion. The *black regions* indicate the 5′ and 3′ untranslated regions and the *white regions* indicate exons. The *solid line* between *open boxes* represents introns. The *triangle* represents the T-DNA. LP and RP as a pair of primers designed in the coding region, and LB is a primer of the T-DNA. Primer orientation is shown with *arrows*. **b** diagnostic PCR of T-DNA inserted in the region of *AtMYB20*. DNA from insertion line of *myb20* (SALK_CS304073) were used

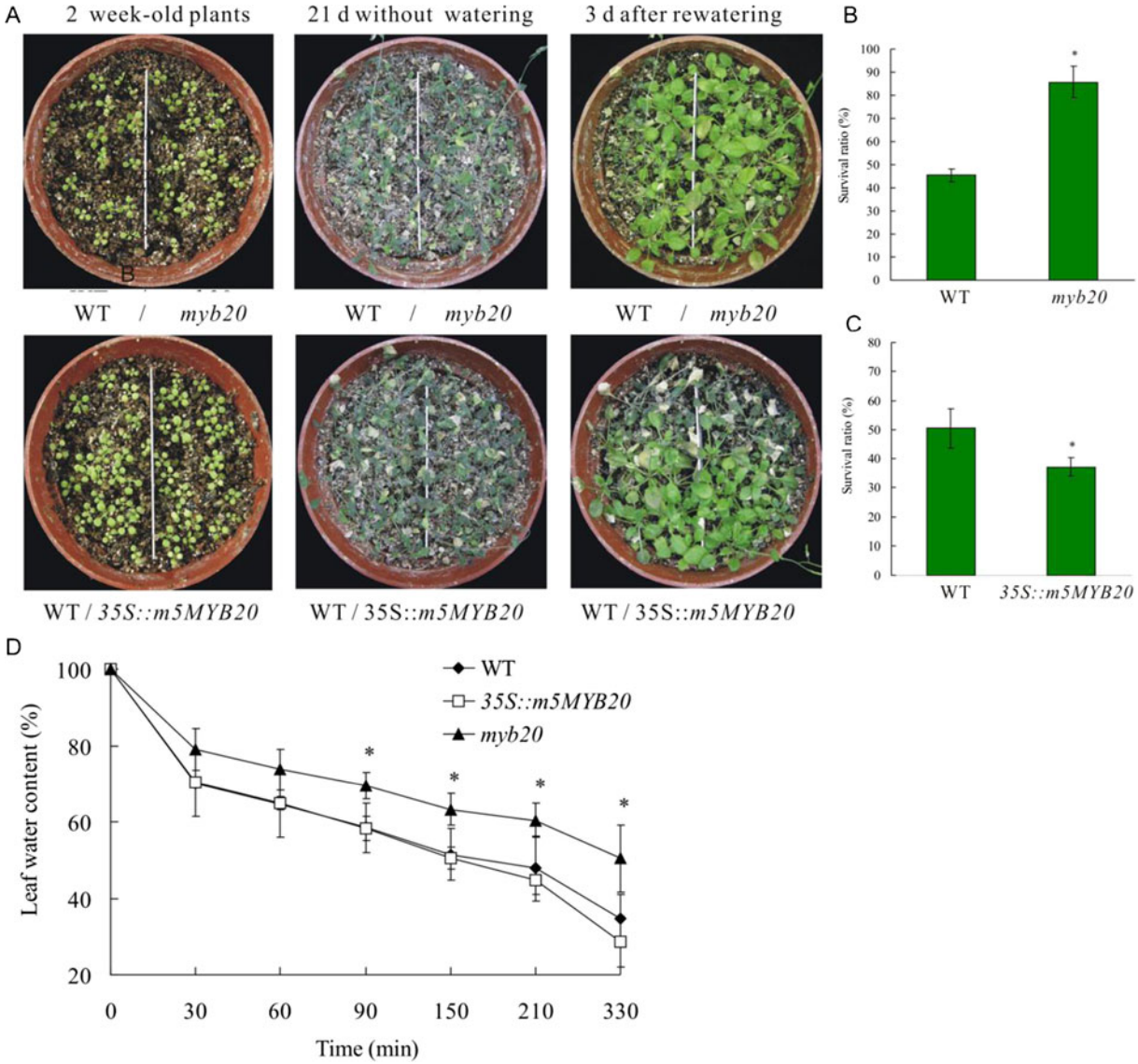


**Fig. 4** Responses of *myb20* and *35S:m5AtMYB* plants to desiccation. **a**/**b**/**c** Two weeks-old wild-type, *myb20* and *35S:m5AtMYB* plants were subjected to desiccation treatment for 21 days, followed by rewatering for 3 days. Desiccation tolerance was expressed as the ability of plants to resume growth when returned to normal conditions following water stress. **d** Measurement of leaf water loss rates. Water loss was calculated as the percentage of initial fresh weight. *Vertical bars* represent the standard deviation of the mean of three treatments. *Asterisks* indicate that mean values are significantly different between *35S:m5AtMYB* or *myb20* plants and WT ($p<0.05$)

## Discussion

With recent advance in high-throughput sequencing technologies and availability of genomic sequences of various plant species, a large number of genes responding at the transcriptional level to abiotic stresses have been identified (Kreps et al. 2002; Khraiwesh et al. 2012; Zhou et al. 2012, 2013). Amongst these, a group of stress-responsive genes encoding transcription factors (*e.g.* MYB, bHLH, bZIP, WRKY, and DREB families) are of particular interest (Shinozaki et al. 2003), because they are involved in many signaling and transcriptional regulatory pathways. R2R3-MYB TFs belong to the largest subfamily of MYB and are involved in various plant abiotic stress responses (Chen et al. 2006; Dubos et al. 2010). Expression of *AtMYB15* was regulated by

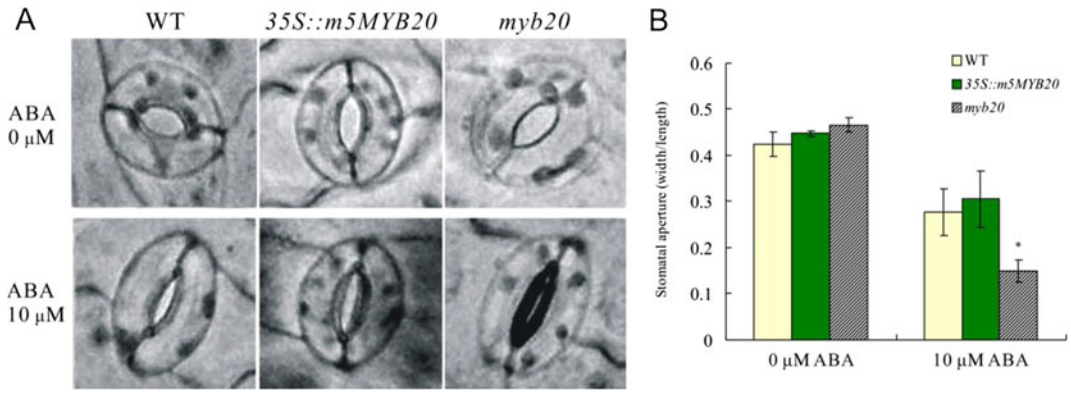


**Fig. 5** Stomatal aperture in leaves of wild-type (WT), *35S:m5AtMYB20* and *myb20* plants treated with ABA. **a** ABA-induced stomata closure. Four week-old mature leaves of WT, *35S:m5AtMYB20* and *myb20* plants were incubated in stomatal opening solution for 2 h and transferred to solutions containing the indicated concentrations of ABA for 2 h. Stomata on the abaxial surface were observed by light microscopy. **b** Measurement of stomatal aperture (the ratio of width to length) after ABA treatment. At least 60 stomatal pores from samples were measured. *Asterisks* indicate that mean values are significantly different between the *35S:m5AtMYB20* or *myb20* plants and WT ($p<0.05$) (**b**)

drought and salinity stresses; *AtMYB15* over-expression plants were able to confer drought tolerance (Ding et al. 2009). Ecotopic expression of the *Chrysanthemum* R2R3-MYB transcription factor *CmMYB2* enhanced tolerance to drought and saline stresses (Shan et al. 2012). Moreover, AtMYB96 transcription factor was shown to mediate abscisic acid signaling during drought stress response in Arabidopsis (Seo et al. 2009). In this study, a R2R3-MYB transcription factor *AtMYB20* has been identified as a regulator of desiccation stress response in Arabidopsis. Like other MYB TFs, *AtMYB20* can be differently regulated by abiotic stresses. However, while *AtMYB20* was induced by high levels of NaCl, its expression was suppressed by desiccation and cold (Fig. 1). This expression pattern with salinity is in accordance with the most recent report; but the *AtMYB20* response to desiccation in this study was slightly different from that to drought stress (Cui et al. 2013).

To identify whether *AtMYB20* was able to regulate plant response to desiccation stress, transgenic lines over-expressing *AtMYB20* were constructed. We applied the *AtMYB20* knock-out mutant *myb20* to identifying *AtMYB20* function. Because *AtMYB20* transcript may be cleavaged by miR858, a cleavage-resistance version to eradicate the interference by miR858 was constructed. Our analysis showed that *35S::m5AtMYB20* plants have a higher level of *AtMYB20* expression than wild-type (4.6 to 6.8-folds, Fig. 2). These transgenic lines plus *myb20* could allow us to demonstrate that *AtMYB20* is critical for plant response to desiccation stress. Whereas *AtMYB20* over-expression resulted in plant sensitivity to desiccation, the *myb20* mutant plants displayed enhanced resistance to water loss.

The phytohormone abscisic acid (ABA) regulates numerous developmental processes and stress responses in plants; under adverse conditions, ABA serves as a signal molecule to sense abiotic stresses; ABA regulates stomatal closure in plants to avoid the water loss during the water stress (Cutler et al. 2010). Heterologous expression in Arabidopsis of *Craterostigma plantagineum* MYB10 has been shown to increase ABA hypersensitivity and enhance drought tolerance (Shan et al. 2012). Similarly, over-expression of miR159-resistant *MYB33* and *MYB101* resulted in ABA hypersensitivity (Reyes and Chua 2007). The present study demonstrated that expression of *AtMYB20* was reduced during the first several hours of ABA treatment (Fig. 1). Our result is in a good agreement with the recent report, in which expression of *AtMYB20* in guard cells was found to be reduced by ABA (50 μM) treatment (Winter et al. 2007; http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi?dataSource=Guard_Cell). While *AtMYB20*-overexpressing plants displayed insensitivity to ABA-responsive stomatal closure, *AtMYB20* knock-out mutation intensified the ABA-promoted stomatal closure in the presence of ABA (Fig. 5). Furthermore, expression of ABA-responsive genes such as *ABI3*, *ABI4*, *ABI5*, *ABF3*, and *ABF4* was more evident in *myb20* plants than in wild-type, whereas expression of these genes in *35S::AtMYB20* plants was depressed. The elevated expression of *ABI3*, *ABI4*, *ABI5*, *ABF3*, and *ABF4* in

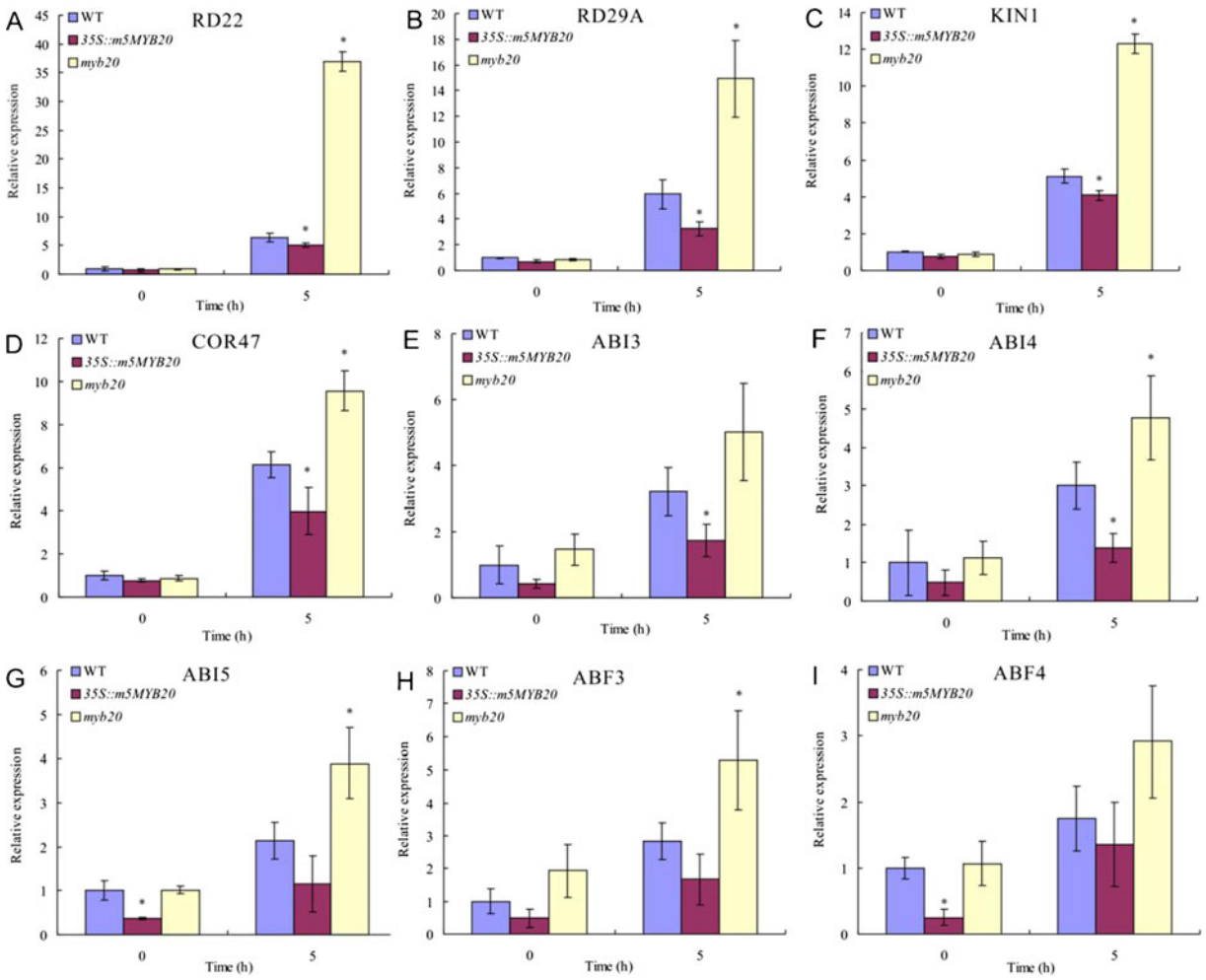


**Fig. 6** Real-time qRT-PCR analysis of desiccation- and ABA-responsive genes in *35S:m5AtMYB20* and *myb20* plants. Two weeks-old wild-type (WT), *35S:m5AtMYB20* and *myb20* seedlings were treated with 100 μM ABA for 5 h, and total RNA was isolated from the seedlings and analyzed by qRT-PCR. The *graphs* indicate the induction fold of the genes with 100 μM ABA as compared with the control (0 μM ABA). *Vertical bars* represent the standard deviation of the mean of three treatments. *Asterisks* indicate that mean values are significantly different between the *35S:m5AtMYB20* or *myb20* plants and WT ($p<0.05$)

*myb20* plants suggests that *AtMYB20* can negatively interact with the ABA synthesis or responsive genes under desiccation stress. In this regard, our result is very similar to the recent study (Cui et al. 2013), in which *AtMYB20* over-expression transgenic lines showed a reduced expression of ABA-responsive genes (*e.g.* ABI1 and ABI2); simultaneously, the salt-induced expression of *AtPP2CA*, a negative regulator of ABA signaling (Kuhn et al. 2006; Yoshida et al. 2006), was found to be significantly depressed in the *AtMYB20* over-expression plants. These results suggest that salt stress would induce *AtMYB20* expression by accumulating ABA, and AtMYB20 in turn may directly bind to the promoters of ABI1 and AtPP2CA to negatively regulate ABA response and ultimately improve the plant salt tolerance. Although the *AtMYB20*-regulated salt and desiccation responses in *AtMYB20* over-expression plants appear different, the two phenotypes should be under the control of ABA signal. The molecular mechanisms underlying the response of plants to desiccation and salt stresses are complicated. Some genes that execute concomitantly positive or negative regulation of both salt and desiccation stress responses have been reported (Shan et al. 2012; Zhao et al. 2013). However, if ABA-dependent signal transduction cascades are involved, genes that depend on ABA signals usually display the contrast regulation of salt and desiccation responses (Zhang et al. 2007; Li et al. 2012). The

*AtMYB20*-regulated desiccation stress response could be also confirmed with *myb20* mutants, in which knock-out of *AtMYB20* resulted in plant more sensitivity to ABA (Fig. 5). In the desiccation experiment, the *myb20* mutant plants always contained a higher level of water in rosette leaves than wild-type (Fig. 4d). Although the *35S::m5AtMYB20* plants showed less water content in leaves than wild-type at the end of the experiment, no significant difference was found between them. It is unclear for the reason, but it was possible that under the desiccation, both wild-type and *35S::m5AtMYB20* leaves underwent a severe lose of water. This could be explained by the observation that guard cells on the leaves of wild-type and *35S::m5AtMYB20* were always open in the presence of ABA (Fig. 5a), which finally may result in an equivalent amount of water loss.

We analyzed the stress-responsive genes *RD22*, *RD29A*, *KIN1* and *COR47*. These genes are up-regulated when plants are exposed to salinity, drought, cold or exogenous ABA (Shinozaki et al. 2003). A group of Ser/Thr protein phosphatase 2C genes, such as *ABI1*, *ABI2*, and *AtPP2CA* were additionally analyzed because these genes were reported to involve the plant salt stress response (Cui et al. 2013). The elevated expression of these genes is considered to be advantageous for plants resistance to abiotic stress (Zhu 2002). In response to ABA, these genes were expressed at higher level in *myb20* mutants than in wild type plants. By contrast, their expression was lower in *35S::AtMYB20* plants. We also found a group of genes (*ABI3*, *ABI4*, *ABI5*, *ABF3*, and *ABF4*) coding for ABA-responsive basic leucine zipper (bZIP) transcription factors had a similar expression pattern in the *35S::AtMYB20* plants and *myb20* mutants. The results indicate that *AtMYB20*-regulated desiccation phenotype is involved in the ABA response and *AtMYB20* is most likely to function as a negative regulator of ABA-mediated stomatal closure.

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